Living and Making Sustainable Choices That Nourish Our Body

A Celebratory Publication
In Honour of

Prof. Musbau Adewumi Akanji
PhD., FNSBMB, FNISEB, FNIMechE (Hon), FAS

And

Prof. Hussein Oyelola Bukoye Oloyede
Ph.D., FNSBMB, FNISEB, FSAN, FIMC, CMC

Edited by
M.T. Yakubu
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Table of Contents

PAGE

Chapter One (BCH UIL Festschrift MAA 001) 1
Toxicological aspect of making environmentally friendly choices and its relevance to healthy living

Chapter Two (BCH UIL Festschrift MAA 002) 10
Honey prevents 3-monochloropropane-1, 2-diol-induced oxidative stress

Chapter Three (BCH UIL Festschrift MAA 003) 20
Ficus sp (fig) as veritable sources of antidiabetic agents: an overview

Chapter Four (BCH UIL Festschrift MAA 004) 36
Evaluation of the effect of coconut oil and high fibre diet on diet-induced hyperlipidemic rats

Chapter Five (BCH UIL Festschrift MAA 005) 58
Nephroprotective properties and effects of aqueous extract of *Ixora coccinea* flower on antioxidant parameters in gentamicin-induced nephrotoxicity in rats

Chapter Six (BCH UIL Festschrift MAA 006) 76
In silico identification of H^+/K^+-ATPase and H. Pylori virulent Cag A and VacA protein inhibitors in ethanolic leaf extract of *Cnidoscolus aconitifolius*

Chapter Seven (BCH UIL Festschrift MAA 007) 95
Neuromodulatory effects of caffeine, vanillin and their combination in aluminum chroride-induced Alzheimer's disease rats

Chapter Eight (BCH UIL Festschrift MAA 008) 118
Modulation of sglt2 and activation of ampk and sirt1 expressions by saponins from *Mormodica charantia*

Chapter Nine (BCH UIL Festschrift MAA 009) 129
Evaluation of quercetin and its sulphonic acid derivatives for antitrypanosomal activities in mice

Chapter Ten (BCH UIL Festschrift MAA 010) 156
Prostate Gland Problems: Functional Foods to the Rescue

Chapter Eleven (BCH UIL Festschrift HOB 001) 196
Gender differences in nutritional response to water-based mud contamination: A rat model study

Chapter Twelve (BCH UIL Festschrift HOB 002) 207
Antioxidant, beta carotene, lycopene, anthocyanin and selected B vitamins levels in sprouted purple fleshed sweet potatoes flour

Chapter Thirteen (BCH UIL Festschrift HOB 003) 219
Cardiovascular diseases: The Roles of nutraceuticals and anti-nutrient phytochemicals

Chapter Fourteen (BCH UIL Festschrift HOB 004) 243
Nutritional/chemical constituents and free radical scavenging potential of yahauri spice

Chapter Fifteen (BCH UIL Festschrift HOB 005) 254
Protective Effects of aqueous extract of Cola acuminata in olanzapine-induced obesity in female Wistar rats

Chapter Sixteen (BCH UIL Festschrift HOB 006) 270
Cucumis sativus fruit homogenate's hepatoprotective effects on Augmentin-induced liver toxicity in Wistar rats

Chapter Seventeen (BCH UIL Festschrift HOB 007) 281
Feed-based-diet of luteolin and astragalin against gamma-irradiation-induced genotoxicity in rats

Chapter Eighteen (BCH UIL Festschrift HOB 008) 293
In vitro antioxidant and antidiarrhoeal activities of hydro-ethanol stem bark extract of guava (*Psidium guajava*) in castor oil-induced diarrhoeal albino rats

Chapter Nineteen (BCH UIL Festschrift HOB 009) 311
Extracts of *Picralima Nitida* Seed and *Tapinanthus Bangwensis* Leaf Effects on Hematology and Serum Electrolytes in Alloxan – induced Diabetic Rabbits

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CHAPTER FOURTEEN

BCH UIL Festschrift HOB 004

NUTRITIONAL/CHEMICAL CONSTITUENTS AND FREE RADICAL SCAVENGING POTENTIAL OF YAHAURI SPICE

Rukayat A. Oyegoke*, Mikhail O. Nafiu and Nafisat Ibrahim
Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515 Ilorin, Nigeria.
*Corresponding author: oyegoke.ra@unilorin.edu.ng

Abstract

The study was aim at evaluating the nutritional/chemical constituent as well as the free radical scavenging potentials of yahauri spice (a blend of spices common among the yahauri community in Northern Nigeria). The blended spices where analyzed for their proximate and mineral composition, phytochemical constituents (Qualitative and Quantitative) and in addition their free radical scavenging properties against Diphenyl-2- picryl-hydrazyl (DPPH). The result of the proximate analysis shows it contains carbohydrate (27.27±0.65), Crude Fiber (34.6±0.73), Crude protein (9.30±0.11), crude lipid (13.14±0.02), total ash (8.48±0.02) and the moisture content was 7.22±0.03, whereas the mineral compositions of the spice include: Magnesium, Iron, Calcium, Zinc, Copper, Nickel and Cobalt with Calcium having highest amount (20.40±0.00). Result of phytochemical screening of the spice revealed the presence of Saponin Alkaloid, Terpenoids Triterpenes and Steroids while the free radical inhibition studies against DPPH shows an increase in inhibition with increase in concentration of the spice. Therefore the study justifies the wide used of the spice among the yahauri community for both culinary and therapeutic purposes owing to its rich bioactive and nutritional composition.

Key words: Yahauri, Spices; Proximate analysis ; Mineral composition; Free radicals

Introduction

Herbs and spices have been used from time immemorial in foods, cosmetics and medicine for the treatment of most illness that affects human (Megha, 2017). They are derived from plants and have wide range of usage, generally, spices are good sources of antioxidants because of which many spices are now used therapeutically for the maintenance of good health (Ranjbar *et al.*, 2021). For instance, while some spices are used as carminatives, and for the improvement of circulatory, hypolipidemic, stomach problems, others are used as stimulants, antispasmodics or for protection against gastrointestinal disturbances (Ye *et al.*, 2021). Herbal infusions have been recognized as important sources of antioxidants which can be consumed on a daily basis as decoctions (Otunola and Afolayan, 2017).

They have several bioactive compounds that behave as antioxidants and have antimicrobial, pharmaceutical, and nutritional properties. The bioactive molecules, including secondary

metabolites secreted by plants, possess a distinct pharmacological property and are also involved in defense against stress (Newman and Cragg, 2012). These plant constituents are known to function against repellents and pathogens called allelochemicals by using several active compounds like flavanols, tocopherols, geraniol-thymol, carvacrol, and benzoic acid which are major antibacterial and antifungal compounds (Adeyemi, 2011). Currently, more studies are focused enormously on the medicinal and nutritional properties of spices. They are a potential source of bioactive compounds such as polyphenols, antioxidants, and essential oils, and antimicrobial chemicals that can deal with the current situation of stress, depression, hormonal imbalance, and metabolic disorders that are common issues in today's lifestyle (Newman and Cragg, 2012). In Nigeria, spices are not only used in culinary but also as galenicals in folk medicine for their putative health benefits. In time past, spices were used primarily for their organoleptic and preservative properties, however recent studies on their medicinal and nutritional properties have come up with exciting results, for instance phytochemicals from spice such as Curcumin (Turmeric), eugenol (Clove) and Capsaicin ((Red pepper) where experimentally shown to control oxidative stress in cell due to their antioxidant properties and their capacity to block the production of oxygen radicals in aerobic metabolism and interfering with signal transduction pathways (Rubió, *et al.*, 2013, Shrivastava *et al.*, 2014).

Yahauri spices is a blend of spices common among the Yahauri community in Northern Nigeria West Africa. It consists of different spices such as Ginger, Garlic, Black pepper, Coriander, African Nutmeg, Cloves, Cinnamon, Anise, Cardamon, Cumin seeds and Fennel seeds. It has a wide usage both as therapeutic and also as flavoring agents in foods, although there is paucity of information about the nutritional/ chemical constituent of the blended spices like its antioxidant potential as claimed by the locals. Hence this study is undertaken to evaluate the nutritional/chemical constituent and also the free radical scavenging potentials of Yahauri spices.

Methods

Plant Materials and Authentication

All the eleven (11) components used for the spice formulation were obtained from Kurmi market, Kano State, Nigeria. These spices were identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin where voucher numbers were obtained as; *Zingiber officinale* (Ginger) UILH/001/2019/1165, *Allium sativum* (Garlic) UILH/002/2019/1209, *Piper nigrum* (Black pepper) UILH/003/2019/1203,*Coriandrum sativum* (Coriander) UILH/004/2019/1390, *Cinnamomum verum* (Cinnamon) UILH/005/2019/1391, *Monodora myristica* (African nutmeg) UILH/006/2019/1257, *Syzygium aromaticum* (Cloves) UILH/007/2019/1107, *Elettaria*

cardamomum (Cardamom) UILH/009/2019/1393, *Illicium verum* (Star anise) UILH/008/2019/1392,*Cuminum cyminum* (Cumin) UILH/010/2019/1394, *Foeniculum officinalis* (Fennel) UILH/011/2019/1395.

Preparation of Yahauri Spice Blend

The eleven components of the spice were dried, pulverized and mixed in different proportions to make up 4kg. The spice blend consists of 1.9 kg of ginger, 240 g of garlic, 180g of black pepper, 220g of coriander seeds, 270g of African nutmeg, 130g of cloves, 150g of cinnamon, 90g of star anise, 80g of cardamom, 430g of cumin seeds and 300g of fennel seeds. This procedure was carried out base on the method used by the locals in Yahauri community.

Proximate Analysis of Yahauri Spice

Proximate analysis was carried out using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005). The following parameter was analyzed: Crude protein, Crude lipid, Total Ash, Moisture, Crude fiber and Carbohydrate.

Preparation of Spices Extract

Extraction was carried out by weighing 100g of the spice blend and infused with 1.5 litres of boiling water (100^0C). The extract was cooled (where necessary) and filtered using a Buchner funnel with Whatman filter paper No.1.

Determination of Secondary Metabolites

Qualitative Phytochemical Screening

Phytochemical constituents of Yahauri spice were determined to identify the various bioactive chemical constituent present in the spice which include: Saponin, Tannin, Flavonoids, Anthocyanins, Steroids, Coumarins, Amino-acids, Glycosides, Alkaloids, Triterpenes, Phlobatamin, Phenolics, Terpenoids and Fixed oil. The method as described by Odebiyi and Sofowora [Odebiyi and Sofowora, 1979; Santhi and Sengottuvel , 2016) was adopted for the study.

Quantitative Screening of Phytochemicals

Saponin

The method described by Santhi and Sengottuvel (2016) with slight modification was used for the analysis of saponin. Briefly, 1g of the finely ground dried sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a magnetic stirrer for 5 hours to ensure uniform mixing. Thereafter filter through a Whatman No. 1 filter paper into a 100ml beaker containing 20ml of 40% saturated solution of MgCO□. The resulting mixture was again filtered to obtain a clear colourless solution. One milliliter of the colourless filtrate was pipetted into a 50ml volumetric flask and 2ml of 5% FeCl□ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to

develop. 0-10ppm saponins standard was prepared from saponins stock solution. The standard solutions were treated similarly with 2ml of 5% FeCIC solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after colour development using a Jenway V6300 spectrophotometer at wavelength of 380nm. Percentage saponin was calculated using the formula:

$$\% \text{ saponin} = \frac{\text{Abs of sample} \times \text{Average gradient} \times \text{Dil factor}}{\text{Weight of sample} \times 10{,}000}$$

Alkaloids

The quantitative determination of alkaloids was done by distillation and titrimetric methods as described by Yeh *et al.*, (2014). Briefly, 2g of finely ground sample was weighed into 100ml beaker and 20mls of 80% absolute alcohol added to give a smooth paste.

The mixture was transferred to a 250ml flask and more alcohol added to make up to 1g of magnesium oxide was then added. The mixture was digested in a boiling water bath for an hour and half under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and re-digested Gallic acid monohydrate, for another thirty minutes with 50ml alcohol after which the alcohol was evaporated. Distilled water was added to replace the lost alcohol. When all alcohol has evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into 250ml volumetric flask, 5ml of Zinc acetate solution and 5ml of potassium ferricyanide solution were thoroughly mixed together to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filtrate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2g of selenium for digestion to a clear colourless solution. The clear colourless solution was used to determined Nitrogen using Kjeldahl distillation apparatus the distillate was back titrated with 0.01N HCl and the titre value obtained was used to calculate the % Nitrogen using the formulae:

%N = Titre value × Atomic mass of Nitrogen× Normality of HCl × 100
Weight of sample (mg)
% Alkaloid = % Nitrogen × 3.26

Where 3.26 is a constant

Steroids

0.05g of sample extract was weigh into a 100ml beaker. 20ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. 1ml of the filterate was pipette into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37°C-40°C for 90 minutes. It was cooled to

room temperature and 10ml of petroleum ether added followed by the addition of 5ml distilled water. This was evaporated to dryness on the water bath. 6ml of Liebermann Buchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4mg/ml was prepared from 100mg/ml stock steroid solution and treated similarly like the sample as above (Maguire, 2004).

% steroid was calculated using the formula:

$$= \frac{\text{Abs. of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Triterpenes

0.50g of sample was weighed into a 50ml conical flask and 20ml 0f 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40ml of 10% Sodium Dodecyl Sulphate (SDS) solution.1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well and allowed to stand for 30 minutes. Standard triterpenes of concentration range 0-5mg/ml was prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510nm (AMC-RSC, 1986).

The percentage of triterpenes was calculated using the formula:

$$= \frac{\text{Abs. of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Glycosides

Determination of glycosides was in line with the AMC-RSC (1986). 10ml of extract was pipette into a 250ml conical flask. 50ml of Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was sieved into a conical flask. 10ml pyridine and 2ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish yellow colour. Glycoside standard of concentration ranging from 0-5mg/ml were prepared from 100mg/ml stock glycoside standard. The series of standards 0-5mg/ml were treated similarly like the sample above. The absorbance of sample as well as standard were read on a spectronic 21D Digital spectrophotometer at a wavelength of 510nm. % Glycoside was calculated using the formula:

$$\frac{\text{Abs of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample}} \times 10{,}000$$

Determination of Mineral Constituents

In line with the method according to AOAC, 2005 and the method described by Bamigboye *et al.* (2020). Analysis of the amount of sodium was carried out by digesting the ash of the sample with perchloric acid and nitric acid, thereafter the reading was taking after appropriate dilution using a flame emission spectroscopy. Other minerals such as Calcium, Magnesium, Zinc, Iron, Copper and Manganese where assayed using an Atomic Absorption Spectrophotometer (AAS) modeled A990 PG Instrument and the value obtained for each mineral in Mg/L analyzed where compared to that of the standard.

Determination of Antioxidant Potentials*In-vitro Assessment of Diphenyl-2- picryl-hydrazyl (DPPH)*

The free radical scavenging properties of the Yahuari spice against 1,1-diphenyl-2-picrylhydraze (DPPH) was determined by a modified method as described by Thakur *et al.* (2021). 1mg of diverse concentrations of the Yahuari spice extract was added to 4 ml (0.004%) methanol solution of DPPH. After 30 minutes' incubation period at room temperature, the absorbance was read against a blank at 517nm. Inhibition of free radical by DPPH in percent (I %) and was calculated using the following formula:

$$I\% = (A_{blank} - A_{sample}/ A_{blank}) * 100$$

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of extract. Tests were conceded in triplicate.

Statistical Analysis

All data were expressed as mean ± standard error of mean (S.E.M). Statistical evaluation of data was performed by using one way analysis of variance (ANOVA), followed by Duncan's multiple range tests for multiple comparisons. The Statistical Package for Social Sciences (SPSS version 20.0 Inc., Chicago, IL, USA) was used for data analysis and values were considered statistically significant at $p<0.005$.

Results

Proximate Analysis of Yahauri spice

Proximate contents of the Yahauri spice powder is represented in table 1.0. Result obtained are from three treatments alongside the standard error of mean (SEM). Crude fiber was found in larger amount (34.6± 0.73) when compared to other components. While Crude moisture had the least amount (7.22±0.03) recorded.

Quantitative phytochemical analysis of yahauri spice

The following phytochmicals: Triterpenes (322.43g), Glycosides (310.69g) and Steroids (208.43g) were

detected in large amount while alkaloids (5.02g) and saponin (0.701g) were detected in lower quantity.

Table 1: Chemical composition of Yahauri Spice

Percentage Composition	Value (100%)
Crude Protein	9.30 ± 0.11
Crude Lipid	13.14 ± 0.02
Total Ash	8.48 ± 0.02
Crude Moisture	7.22 ± 0.03
Crude Fibers	34.6 ± 0.73
Carbohydrate	27.27 ± 0.65

Table 2: Quantitative phytochemicals screening of studied Yahauri spice

Phytochemical	(Mg/100g)
Saponin	0.701
Steroids	208.43
Glycoside	310.69
Triterpenes	322.43
Alkaloids	5.02
Terpenoids	66.92

Mineral Composition of Yahauri spice

The Yahauri spice contained considerate large amount of calcium as revealed in table 4.0. No trace of Sodium and Nickel was recorded. Nevertheless, Iron, Magnesium and Manganese were obtained in relatively small amount and the value for Zinc, Copper and Cobalt were below 1.0 mg/L.

Table 3: Mineral Composition of Yahauri spice

Minerals	Value (mg/L)
Mg	1.20 ± 0.00
Mn	4.15 ± 0.02
Cu	0.43 ± 0.00
Zn	0.10±0.02
Na	0
Ca	20.40 ± 0.00
Fe	4.00 ± 0.10
Ni	0
Co	0.34 ± 0.01

Antioxidant potentials of Yahauri spice

Figure 3.0 shows the graphical representation of the DPPH antioxidant report against Yahauri spice powder. The percentage inhibition of both the spice and the standard (Ascorbic acid) are represented in the graph. The concentration of the spice and standard ranged from 50 mg/ml to 10 mg/ml, also the per cent inhibition increases as the concentration of the spice and standard increases.

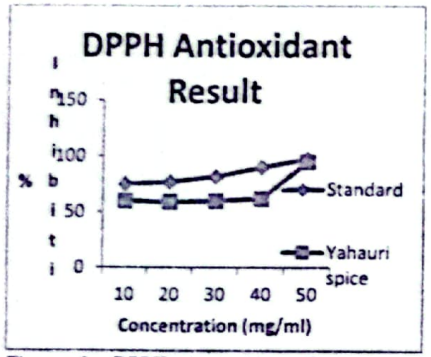


Figure 1: DPPH Antioxidant Result of Yahauri Spice

Discussion

Spices are known to be rich in bioactive substance with useful and beneficial properties in food, cosmetics and pharmaceutical industries. The present study was targeted at evaluating the nutritional/chemical constituent and free radical scavenging potentials of Yahauri spices. Proximate analysis of the spice shows a high content of carbohydrate (27.27±0.65), crude fibers (34.6± 0.73), crude lipid (13.14±0.02) and crude protein (9.30±0.11). While the moisture content (7.22±0.03), total ash (8.48±0.02) recorded a low amount. The high fiber content recorded in the Yahauri spice implies it is a good source of dietary fiber, which from studies it was reveal that fiber content in food aids in digestion prevent constipation and manage heart disease (Kaefer and Milner, (2011), Mann (2011), Opara and Chohan (2014).

The result of the qualitative and quantitative results of the phytochemical constituents of the Yahauri spice powder shows the present of Saponin, steroids, glycosides, alkaloids, terpenoids, triterpenes and fixed Oil. The presence of these phytochemicals proved the claim on the use of the spice for medicinal purposes. Forinstance Alkaloids are made up of heterocyclic nitrogen that has been shown to exhibit antimalarial, antihypertensive, antiarrhythmic, and anticancer properties (Ogbunugafor, 2017). Alkaloids have also been reported to act as CNS stimulant and powerful analgesics (Ashok and Upadhyaya, 2012). Likewise Saponins have been reported to have antimalarial effect (Besong, 2016) while Cardiac glycosides are proven to be important in the management of cardiovascular diseases (Rubió *et al.*, 2013) Terpenes and terpenoids are known to possess anti-inflammatory, antioxidant, anticancer, antiseptic, antiplasmodial, astringent, digestive, diuretic, and many other properties (Cox-Georgian *et al.*, 2019).

For a diet to be balance, it must contain adequate minerals necessary for the building and development of the body. Analysis of the mineral content of Yahauri spice revealed the presence of vital minerals (Table 4.0). Notable among which is calcium which was detected in high amount (20.40 mg/L). Studies have revealed that calcium is required in diets for strong bones, healthy teeth and in addition plays a vital role in blood clotting (Beto, 2015, Newman, 2020). The Yahauri spice studied shows the availability of magnesium in considerate amount (1.20 mg/L). The Yahauri spice studied shows

the availability of magnesium in considerate amount (1.20 mg/L). Magnesium is a component of many enzymes in the body and it plays a critical role in bone formation and in maintaining bone density. It also helps the body effectively use the building blocks of strong bones, including the nutrients calcium and Vitamin D (Gröber *et al.*, 2015). Other minerals, Copper, Manganese, Iron and Cobalt are seen in their respective quantities. Cobalt and Iron are needed for healthy blood system. The availability of Iron (4.00 mg/L) and Cobalt (0.34 mg/L) signifies a rich mineral profile of the Yahauri spice. The body utilized iron in the production of hemoglobin and myoglobin (Abbaspour *et al.*, 2016). The minerals presence in Yahauri spice makes it a potential supplements to be utilize as feed or other culinary purpose owing to its rich mineral contents. However, Sodium and Nickel were not detected among the studied minerals.

DPPH radical scavenging assay is commonly used to test the ability of an extract to act as free radical scavengers which in-turn shows the antioxidant potential of the extract (Alam, 2013). The yahauri spice demonstrated a concentration-dependent ability to scavenge free radicals, it compared favorably with that of the standard (Ascorbic acid) used as reference. The radical scavenging activity of the spice extract may be attributed to its phytochemical constituents with a functional hydroxyl group acting as hydrogen donor, this agree with the findings of Lima *et al.*, (Lima , 2015) that most phytochemicals with hydroxyl group as the ability to scavenge free radicals.

Conclusion

In conclusion, phytochemical constituent of the yahauri spice discovered in the course of the study include Saponin, Alkaloids, Triterpenes, Steroids, and Terpenoids, hence the study justify to a great extend the use of yahauri spices for culinary as well as for therapeutic purpose owing to the bioactive and mineral constituents present in the blended spices, likewise the free radical scavenging properties of the spice by its inhibition against DPPH makes it a good source of antioxidant that are of benefits to human health.

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